Figure 5. NleB O-GlcNAcylates GAPDH

(A) Detection of GAPDH O-GlcNAcylation by NleB using 5-carboxytetramethylrhodamine (TAMRA).

(B) Detection of GAPDH O-GlcNAcylation by NleB using α-O-GlcNAc antibody (110.6).

(C) GAPDH O-GlcNAcylation as a function of [UDP-GlcNAc]. NleB- or NleC-FLAG was incubated with GAPDH and UDP-GlcNAc. Proteins were analyzed by SDS-PAGE and immunoblotted for the O-GlcNAcylation using α-GlcNAc antibody.

(D) GAPDH O-GlcNAcylation during bacterial infection. HeLa cells were infected with *C. rodentium* WT or Δ*nleB* for 1 or 3 hr. Cell lysates were immunoprecipitated with α-GAPDH antibody and analyzed for O-GlcNAcylation. Asterisks indicate other proteins detected in GAPDH immunoprecipitates more heavily O-GlcNAcylated in the presence NleB.

(E) NleB(AAA) does not O-GlcNAcylate GAPDH. HeLa cells were cotransfected with NleB, TRAF2, and GAPDH plasmids for 60 hr and treated with 20 μM TMG for 8 hr ± TNF (20 s). Cell lysates were immunoprecipitated for TRAF2-FLAG or GAPDH-Myc. Cell lysates were also immunoblotted for total protein O-GlcNAcylation.

(F) Purified GAPDH mutant proteins were incubated with NleB-FLAG and UDP-GlcNAc. GAPDH O-GlcNAcylation was analyzed using α-GlcNAc antibody. GAPDH and NleB proteins were analyzed using IRDye blue protein staining. The rightmost lanes of the 'GAPDH' and 'NleB' gels served as loading controls and were not assayed for GAPDH O-GlcNAcylation.

(G) FLAG-NleB WT and mutants were purified and incubated with WT GAPDH. Samples were analyzed using SDS-PAGE and immunoblotted with α-GlcNAc and α-GAPDH antibodies.

See also Figure 3.

Placental Malaria: From Infection to Malfunction

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Differential Responses of Immune Cells to Type I Interferon Contribute to Host Resistance to Viral Infection

Thomas Baranek, Thien-Phong Vu Manh, Yannick Alexandre, Muhammad Ahmad Maqbool, Joaquin Zacarias Cabeza, Elena Tomasello, Karine Crozat, Gilles Bessou, Nicolas Zucchini, Scott H. Robbins, Eric Vivier, Ulrich Kalinke, Pierre Ferrier, and Marc Dalod*
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